

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A high sensitivity method of multiple microorganism detection which is a method for detecting two or more microorganisms in foods having different properties ~~in foods, with high sensitivity comparable or even superior to official methods,~~ by amplifying a plurality of target genes with a single PCR reaction tube and analyzing the same, comprising the following steps:
(A) a step for extracting DNA of the target microorganisms to be detected, by treating at least with a lytic enzyme and/or bacteriocin having lytic activity, a surfactant and a protein denaturant; and
(B) a step for performing Multiplex PCR by mixing a primer specific to the target microorganisms to be detected.
2. (Original) The method of multiple microorganism detection according to claim 1, wherein a step to culture microorganisms under a culture condition where 1 CFU/100 g microorganisms become 10^3 CFU/ml or more after 24 h of culture, is included prior to the step of extracting DNA of the target microorganisms to be detected.
3. (Original) The method of multiple microorganism detection according to claim 1 or 2, wherein the two or more microorganisms with different properties comprise *Listeria monocytogenes*.
4. (Original) The method of multiple microorganism detection according to claim 3, wherein the specific primer is a primer consisting of base sequences shown by SEQ ID Nos: 5 and 6.

5. (Withdrawn) The method of multiple microorganism detection according to claim 1 or 2, wherein the two or more microorganisms with different properties comprise pathogenic *Escherichia coli* O157.
6. (Withdrawn) The method of multiple microorganism detection according to claim 5, wherein the specific primer is a primer consisting of base sequences shown by SEQ ID Nos: 1 and 2, or SEQ ID Nos: 7 and 8.
7. (Withdrawn) The method of multiple microorganism detection according to claim 1 or 2, wherein the two or more microorganisms with different properties comprise *Salmonella* spp.
8. (Withdrawn) The method of multiple microorganism detection according to claim 7, wherein the specific primer is a primer consisting of base sequences shown by SEQ ID Nos: 3 and 4, or SEQ ID Nos: 9 and 10.
9. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the microorganisms are cultured in a culture condition where the pH after culture becomes 5.1 or more.
10. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the microorganisms are cultured in a medium with glucose concentration of 0.15% or less, and/or in a medium with concentration of phosphate-buffer solution of 50 mM or more or in a medium with a buffer ability similar as that with concentration of phosphate-buffer solution of 50 mM or more.
11. (Currently amended) The method of multiple microorganism detection according to claim 1 or 2, wherein the extraction is performed after treating with a lytic enzyme and/or ~~baeteriosin~~ bacteriocin having a lytic activity, further treating with a surfactant and a protein denaturant, removing insoluble fractions by centrifugation, and by depositing DNA by alcohol precipitation.
12. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the lytic enzyme is Achromopeptidase and/or Lysozyme.

13. (Currently amended) The method of multiple microorganism detection according to claim 1 or 2, wherein ~~bacteriosin~~ bacteriocin having lytic activity is Enterolysine.
14. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the surfactant is ethyleneoxide condensate of sorbitan monolaurate.
15. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the protein denaturant is Guanidine isothiocyanate.
16. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein Multiplex PCR is performed by combining DNA consisting of base sequences shown by SEQ ID NOs: 1 to 6 at a total concentration of 750 nM or less as a primer.
17. (Withdrawn) The method of multiple microorganism detection according to claim 1 or 2, wherein Multiplex PCR is performed by combining DNA consisting of base sequences shown by SEQ ID NOs: 5 to 10 at a total concentration of 750 nM or less as a primer.
18. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the food is edible meat or processed meat product.